PCR Protocol

PCR Reaction:

Your forward primer (10 μM)***	2.5 μl
Your reverse primer (10 μM)***	2.5 μl
Template DNA	0.5 μl
2x Phusion Master Mix	25 μl
Water	19.5 μl
TOTAL	50 μl

*** Your stock primers are 100 μM. You must make a tube of diluted primer to use for your cloning!***

1. Mix the above reactions in PCR tubes on ice. Make sure to mix well since the enzyme is viscous and sinks to the bottom.

2. Put in the thermocycler for the following cycle:

Initial Denaturation	98°	30 s
35 cycles of		
Denaturation	98°	10 s
Annealing	55°	20 s
Extension	72°	1 m (15-30s/kb)
Final Extension	72°	5 min
Hold	4°	forever

3. Run 5 μl sample on 1% agarose gel to verify products.